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Reprinted from
Vol. 6, No. 6, 1961/62 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

A CHEMICAL BASIS FOR THE SENSITIZATION OF BACTERIA TO ULTRAVIOLET LIGHT BY INCORPORATED BROMOURACIL *

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Received December 13, 1961

The incorporation of the thymine analog, 5-bromouracil (BU), into the deoxyribonucleic acid (DNA) of bacterial and mammalian cells results in an increased sensitivity of these cells to the lethal effects of ultraviolet light (Greer, 1960; Djordjevic and Szybalski, 1960; Kaplan, Smith and Tomlin, 1961 a and b). Beukers and Berends (1961) have reported that the ultraviolet (U.V.) irradiation of frozen aqueous solutions of thymine brings about the formation of a dimer. This dimer is also obtained when DNA is irradiated in vitro and offers a possible chemical basis for the action of ultraviolet light on DNA. The formation of a stable dimer would presumably interfere with the coding function of DNA. The formation of the thymine dimer has also been demonstrated to occur when bacteria were irradiated in vivo (Wacker, Dellweg and Weinblum, 1960). It therefore seemed reasonable to postulate that BU might exhibit a similar but enhanced photochemical response to ultraviolet light as the basis for its radiation sensitizing properties. Moore and Thomson (1955) have reported that BU is somewhat more sensitive than thymine when irradiated in solution. The data to be presented show that the BU which is incorporated into the DNA of E. coli B/r is more sensitive in vivo than is thymine. This is measured by the disappearance of the parent compound and the concomitant appearance of photoproducts with increasing doses of ultraviolet light.

*This investigation was supported by Grant #C-2896 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

EXPERIMENTAL AND RESULTS:

For in vitro studies, solutions of the several purines and pyrimidines (0.5 mg/ml. water) were frozen and irradiated in a freezer (-20°) with a Mineralight lamp (254 mμ) at 5 cm distance (5400 ergs/mm^2/min) for up to 60 minutes. Where indicated, equal volumes of the bases were mixed and irradiated. The solutions were chromatographed in butanol/acetic acid/water (200/30/75) and then surveyed for ultraviolet absorption and radioactivity. Bromouracil-2-C-14 alone forms no detectable photochemical products when it is irradiated under the same conditions in which thymine-2-C-14 is converted to the thymine dimer in 86% yield. No photoproducts are formed when an equal weight mixture of thymine-2-C-14 and BU (and the reciprocal radioactive mixture) is irradiated. Thus, BU (and also adenine, but not uracil or cytosine) inhibits in vitro thymine dimer formation presumably by disturbing the orientation of the thymine molecules. However, if bromouracil-2-C-14 and cytosine are mixed and irradiated in frozen aqueous solution, three photoproducts of BU are formed. Since cytosine-2-C-14 undergoes no detectable chemical change when irradiated singly or mixed with either uracil, thymine, adenine or BU, it would appear to be functioning (when mixed with BU) only as an inert agent which allows the favorable packing of BU so that it can undergo photochemical interactions when activated by ultraviolet light. All three of these photoproducts of BU show an increase in optical density at 260 mμ on reirradiation in alkaline solution, a response which is characteristic of the thymine dimer (Beukers and Berends, 1961). When bromouracil-2-C-14 and uracil (and the reciprocal mixture) are irradiated, four to six photoproducts are formed of which only about half involve uracil. The presence of uracil thus not only allows favorable packing for interaction of the BU molecules, but also for probable cross reactions with uracil. One of these photoproducts arises solely from BU yet no longer contains bromine and maintains a strong U.V. absorption which differs from that of uracil or BU. It may be formed in a manner similar to that described by Wolf and Kharasch

(1961) for the formation of biphenyl by the ultraviolet irradiation of a mixture of iodobenzene and benzene. Uracil irradiated alone yields two major photoproducts.

When E. coli labeled either with thymine-2-C-14 or bromouracil-2-C-14 are irradiated with ultraviolet light one can determine the sensitivity of the labeled compounds by following their rate of disappearance (and concomitant appearance of photoproducts) with increasing doses of ultraviolet light. The experimental details and results of such experiments are given in Fig. 1. The data for thymine were obtained from three independent experiments while those for bromouracil came from four experiments. The lines were fitted by regression analysis and the standard error of the slope when expressed as the percent of the slope was 5.4% for thymine and 4.7% for bromouracil. From the slope ratio of the two lines it is calculated that, in vivo, BU is 1.9 times more sensitive to irradiation by ultraviolet light than is thymine.

The disappearance of parent compound due to the action of ultraviolet light is accompanied by the appearance of photoproducts. The data for the appearance of photoproducts with increasing doses of ultraviolet light are taken from the experiments described in the legend to Figure 1. and are plotted in Figure 2. The photoproducts are identified by their R_f in butanol/acetic acid/water (200/30/75). The curves are plotted from the slopes calculated by regression analysis. The standard error of the slope expressed as the percent of the slope was 3.2% for the thymine dimer, and for the BU photoproducts, listed in increasing order of R_f value, 5.7%; 11.7%; 10.9%; 18.9% and 13.3%, respectively.

Thus, the greater rate of photochemical alteration of BU relative to thymine in vivo is accompanied by the formation of five photoproducts whereas thymine forms mainly one. The presence of two adjacent thymine molecules in a strand of DNA is considered necessary for the in vivo formation of a thymine dimer. A dimer of BU could presumably be formed by a similar mechanism. With BU, however, we have the added complication of

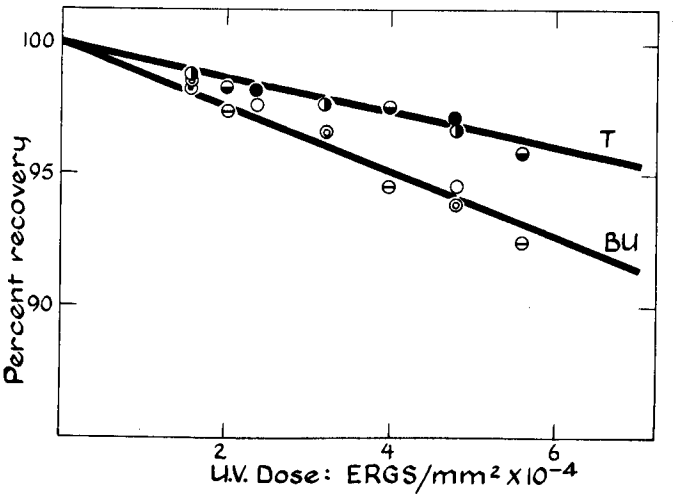

Figure 1. The Relative Sensitivity of Thymine and Bromouracil to Alteration by Ultraviolet Light when Incorporated into the DNA of E. coli B/r and irradiated in vivo.

E. coli B/r were inoculated into 100 ml. of mineral medium containing 2% sulfanilamide (Kaplan, Smith and Tomlin, 1961, b), to which was added 1 mg (25 μc) of thymine-2-C-14 (T) or bromouracil-2-C-14 (BU), and incubated at 37°C for 24 hours. The replacement of thymine by BU under these conditions was around 70%. The cells were harvested, divided into 4 portions (approximately 3 x 10^{10} cells), suspended in 10 ml. phosphate buffer and irradiated with shaking under an unfiltered General Electric germicidal lamp (G8T5) whose output was 13.3 ergs/mm²/sec at 43 cm distance. Cells were irradiated for various lengths of time, harvested, washed 2x in 5% trichloracetic acid and 2x in ethanol-ether (3:1), hydrolyzed in trifluoroacetic acid (Dutta, Jones and Stacey, 1956), and chromatographed in butanol/acetic acid/water (200/30/75). Chromatograms were photographed (Smith and Allen, 1953) to locate the ultraviolet absorbing spots and run through a strip scanner for the detection of radioactive areas. The radioactive areas were cut out, eluted and re-counted in a liquid scintillation counter and the amount of parent compound and photoproducts was calculated as percent of the total radioactivity. In the experiments with thymine, more than 99% of the incorporated radioactivity could be recovered as thymine in the unirradiated controls. In the experiments with BU (radiopurity > 99%), more than 90% of the total incorporated radioactivity was present as bromouracil in the unirradiated controls; less than 10% appeared in products which would have been expected if the bromouracil had been debrominated (see also Wacker, Kirschfeld and Weinblum, 1960).

the removal of bromine atoms by photolysis. It would be possible therefore to obtain not only a dimer of BU, but the single and double debrominated products as well. If the cyclobutane ring structure of the dimer is the site of action of the photoreactivating enzyme (Rupert, 1960), then thymine of course would be liberated when a thymine dimer (in DNA) is repaired. The

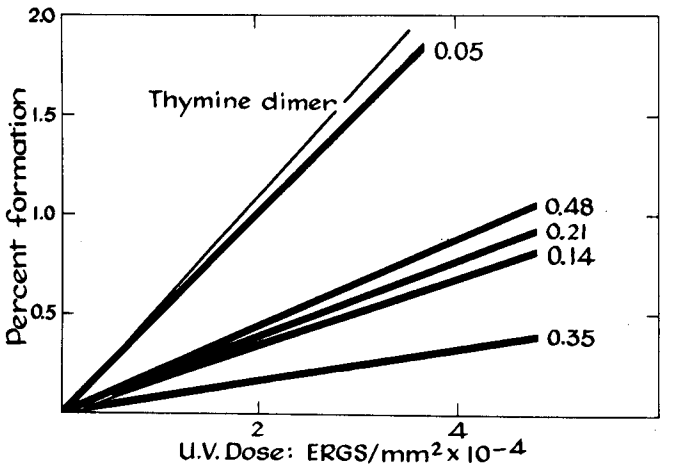

Figure 2. The Number and Rate of Appearance of Photoproducts of Thymine and Bromouracil Incorporated into the DNA of E. coli B/r and Irradiated with Ultraviolet Light in vivo.

The data are taken from the experiments described in the legend to Figure 1. and are further described in the text. The numbers that identify the lines are the R_f values for the BU photoproducts chromatographed in butanol/acetic acid/water (200/30/75). The R_f of thymine is 0.60; thymine dimer, 0.24 and bromouracil, 0.61.

photoreactivation of a debrominated BU dimer (in DNA) would yield uracil and thus would not give rise to a physiologically competent DNA molecule.

In summary, these studies show that at least one explanation for the enhancement of bacterial sensitivity to ultraviolet light by incorporated BU is its greater photochemical lability in vivo relative to thymine. This is evidenced both by a greater rate of photochemical alteration (1.9 times greater than thymine) and also by a greater number of photochemical products formed. A further examination of the photoproducts of BU obtained both in vivo and in vitro will be the subject of a subsequent publication.

ACKNOWLEDGMENTS

I would like to acknowledge the technical assistance of Mrs. Mary E. O'Leary and to thank Dr. Henry S. Kaplan for valuable suggestions during the preparation of this manuscript.

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